A proposal for categorizing UVCBs for aquatic ecotoxicity testing

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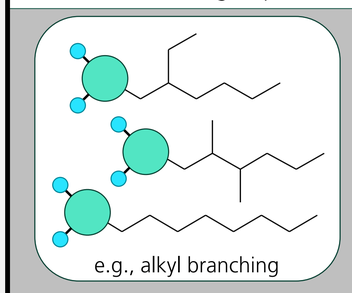
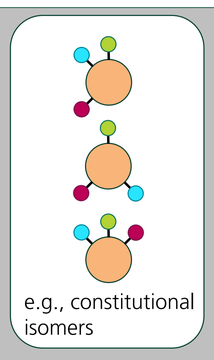
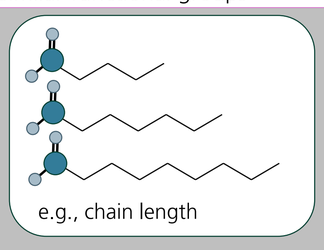
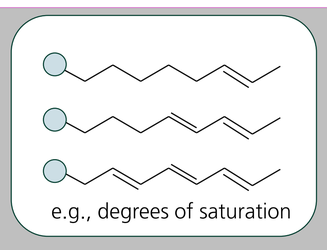
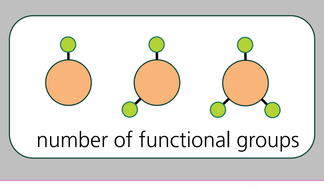
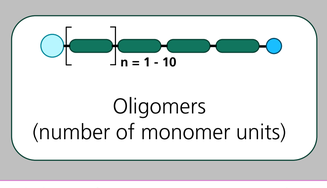
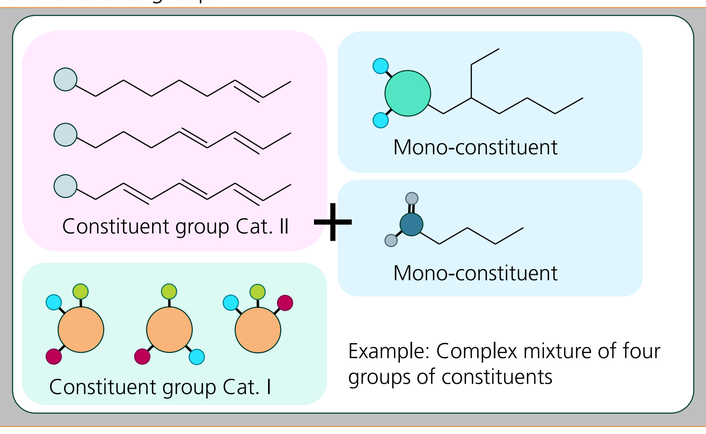
Introduction

UVCBs (unknown or variable composition, complex reaction products or of biological materials) are an important group of substances registered under the REACH regulation, estimated to a share of 20-25% of registered substances [1]. UVCBs are considered difficult-to-test substances (DTTS) according to OECD Guidance 23 document [2] and comprise of a multitude of constituents, which are often unknown and may differ significantly in their physical-chemical properties. The UVCBs often comprise further challenging properties, such as poor water solubility, hydrophobicity, volatility, fast degradation in the test system or others. This complicates testing in environmental risk assessment studies, such as in aquatic ecotoxicological studies.

The existing guidelines often do not reflect these difficulties, and thus more guidance is needed for testing and data evaluation. Therefore, a proposal was developed to further categorize UVCBs. For the respective categories, analytical considerations, application approaches according to OECD GD 23 [2] as well as options for data evaluation are presented. These considerations support the decision whether testing of the whole substance or representative constituents of the test item may be deemed, according to the concept developed by Déglin et al. 2026 [1]. Thereby, this proposal aims to streamline and facilitate the testing of UVCBs for a sound environmental risk assessment.

	UVCB Category I	UVCB Category II	UVCB Category III
Categorization based on chemical similarity	➢ **Mixtures of isomers** Defined by: • Almost identical physical-chemical properties • Same molecular weight • Same functional groups	➢ **Homologous series (e.g., Carbon chain lengths)** ➢ **Oligomers** ➢ **Constitutional similarity** Defined by: • Similar physical-chemical properties often according to homologous series • Different molecular weights • Same/similar functional groups	➢ **Complex mixtures of different constituents** ➢ **Can consist of multiple mono-constituents and/or multiple UVCB sub-groups of Category I or Category II** Defined by: • Largely different physical-chemical properties • Different molecular weights • Different functional groups
	 	   	
Analytical considerations	• Sometimes chromatographically irresolvable or indistinguishable by detectors (e.g., due to same mass, UV/VIS absorption, fluorescence) • Often same concentration-response relation in detection system → May be quantified within one analytical method as a sum parameter or with less specific detection (e.g., UV/VIS, fluorescence, FID)	• Chromatographic retention typically in line with homologous series or number of monomers • Mostly similar concentration-response in detection systems due to same functional groups (sometimes slightly increasing or decreasing response over the homologous series) → May be quantified with one analytical method as individual constituents. Less specific detection methods may be used when chromatographic separation is ensured (e.g., UV/VIS, fluorescence, FID)	• Often completely different concentration-response relation in detection systems due to different functional groups • For individual substance groups within the UVCB, separate analytical approaches according to Categories **I** or **II** may be applied → Different substance groups require use of highly specific analytical methods. Different analytical methods for each substance group may be required (e.g., LC-MS, GC-MS)
Application in ecotoxicological tests	• Constituents behave similarly, and test application likely does not alter the composition of this UVCB No other difficult-to-test properties apply (i.e., all constituents are fully soluble and stable in the test) ➢ Treatment like a mono-constituent substance Other difficult-to-test properties apply ➢ Treatment as a mono-constituent substance, consideration of OECD GD 23 for test application (e.g., by water accommodated fractions (WAF) or passive dosing)	• Constituents behave rather similarly; however, many physical-chemical properties change according to homologous series (e.g., tendence to higher lipophilicity with increasing carbon chain length) • Biological and chemical stability of the constituents in the test system can vary according to the homologous series No other difficult-to-test properties apply ➢ Behavior needs to be confirmed in preliminary analytical experiments for all relevant constituents or at least both ends of a homologous series or all constituents where possible Other difficult-to-test properties apply ➢ Consideration of OECD GD 23 for test application; behavior needs to be confirmed for all relevant constituents or at least both ends of a homologous series in preliminary analytical experiments ➢ The test item may be altered in its composition if stable test concentrations cannot be reached for all constituents	• Groups of constituents behave differently with vastly different physico-chemical properties • Within each group of constituents there may be similarity according to Cat. **I** or **II** • Biological and chemical stability in the test system likely differs between groups of constituents No other difficult-to-test properties apply for each groups of constituents and within each group ➢ Behavior needs to be confirmed for all groups of constituents and within each group individually Other difficult-to-test properties apply to at least one group of constituents ➢ Consideration of OECD GD 23 for test application ➢ Each group may have its own optimum for test application, which may prevent full application of the test item. In this case care must be taken to capture the constituents that are most toxic, if data on toxicity is available ➢ The test item may be altered in its composition if stable test concentrations cannot be reached for all constituents
Data evaluation	➢ **Evaluation based on a sum parameter or based on concentrations of individual constituents** (if specifically analyzable) Note: in some cases, certain isomers may have a specific toxicity. In this case, a differentiation by chiral methods might be required. Evaluation can be based on the most toxic compound (if known) **I.a)**: Exposure levels <u>within 80-120% over test duration</u> → Evaluation based on nominal loadings **I.b)** Exposure levels <u>outside 80-120% over test duration</u> → Evaluation based on measured concentrations	➢ **Evaluation based on concentrations of individual constituents** **II.a)** Exposure levels <u>within 80-120% for all constituents over test duration</u> Evaluation based on nominal loadings **II.b)** Exposure levels <u>outside 80-120% similarly for all constituents over test duration</u> Evaluation based on the sum of measured concentrations of all constituents **II.c)** Exposure levels <u>outside 80-120% and different for constituents over test duration</u> →In all cases, evaluation based on nominal loadings acc. to OECD GD 23. →Additional information: Evaluation may be based on the sum of measured concentrations of all constituents ("average approach") →Additional information: Evaluation may be based on the recovery of both ends of the homologous series ("worst- and best-case approach"). If toxicity data on individual constituents are known, the most toxic constituents may be considered instead.	➢ **Evaluation based on concentrations for all groups of constituents** **III.a)** Exposure levels <u>within 80-120% for all constituents over test duration</u> Evaluation based on nominal loadings **III.b)** Exposure levels <u>outside 80-120% similarly for all constituents over test duration</u> Evaluation based on the sum of measured concentrations of all constituents **III.c)** Exposure levels <u>outside 80-120% and different for constituents over test duration</u> →In all cases, endpoints should be given based on nominal loadings acc. to OECD GD 23. →Additional information: Evaluation may be based on the recovery of individual groups of constituents. If toxicity data on individual constituents or groups thereof are known, the most toxic constituents may be considered instead.

<u>Whole substance approach [1]</u>
The substance properties are within certain ranges that allow testing of the whole substance

(in special cases)

<u>Representative constituent approach [1]</u>
Testing of fractions of the UVCB or individual groups of constituents may be deemed to identify drivers for toxicity. Screening approaches or NAM methods may be used as preliminary tests and to avoid additional vertebrate testing. Literature data or read-across might also be added.

Conclusion

- A concept was developed to classify UVCBs into three categories based on their chemical complexity and composition
- The categorization streamlines considerations regarding chemical analysis, application approaches, and data interpretation
- Depending on the category, different approaches such as a whole substance approach or the separate testing of constituents or fractions of the UVCB are necessary
- More research as well as regulatory guidance is needed to further refine test strategies and options for data evaluation, not only in aquatic ecotoxicity but also in other study types of environmental risk assessment

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References:

[1] Déglin et al. 2026 [DOI: 10.1093/inteam/vjaf200]
[2] Guidance document on aqueous-phase aquatic toxicity testing of difficult test chemicals; series on testing and assessment; No. 23 (Second Edition)

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